

FORM PTO-1390

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NUMBER SMAR-0013

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)

10/019341

INTERNATIONAL APPLICATION NO.
PCT/CA00/00762INTERNATIONAL FILING DATE
23 June 2000 (23.06.00)PRIORITY DATE CLAIMED
24 June 1999 (24.06.99)

TITLE OF INVENTION LPL VARIANT THERAPEUTICS

APPLICANT(S) FOR DO/EO/US Michael R. Hayden and John J. P. Kastelein

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

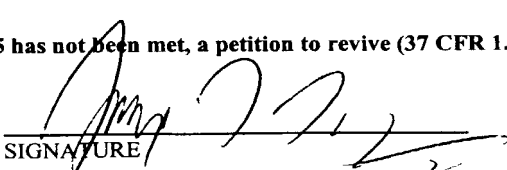
1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☒ has been transmitted by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) 35 U.S.C. 371(c)(4).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment (canceling claims 1-35 and adding new claims 35-57 (10 pages).
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Copy of publication number WO 01/00220A2, including the International Search Report; Copy of Written Opinion (8 pages); Copy of Amendment Under Article 34 (4 pages); International Preliminary Examination Report (9 pages); Check in the amount of \$944.00; Postcard

EL695382365US

EXPRESS MAIL Mailing Label No. EL695382365US
Date of Deposit: December 21, 2001

U.S. APPLICATION NO. 10/019341 <small>(if known 37 CFR 1.492(a)(1) - (5))</small>		INTERNATIONAL APPLICATION NO. PCT/CA00/00762		ATTORNEY DOCKET NUMBER SMAR-0013	
17. <input checked="" type="checkbox"/> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... \$1,040.00 International preliminary examination fee (37 CFR 1.482 not paid to USPTO but International Search Report has been prepared by the EPO or JPO..... \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... \$740.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)..... \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT =</div>				<u>CALCULATIONS</u> <u>PTO USE ONLY</u>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <u> 20 </u> <u> 30 </u> months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
Claims	Number Filed	Number Extra	Rate		
Total claims	23 - 20 =	3	X \$18.00	\$54.00	
Independent Claims	2 - 3 =	0	x \$84.00	\$0	
Multiple dependent claims(s) (if applicable)			+ \$280.00	\$	
TOTAL OF ABOVE CALCULATIONS =				\$54.00	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				\$	
SUBTOTAL =				\$944.00	
Processing fee of \$130.00 for furnishing the English translation later than <u> 20 </u> <u> 30 </u> months from the earliest claimed priority date (37 CFR 1.492(f)).				+	\$
TOTAL NATIONAL FEE =				\$944.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				+	
TOTAL FEES ENCLOSED =				\$944.00	
				Amount to be:	
				refunded	\$
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a. <input checked="" type="checkbox"/> A check in the amount of \$944.00 to cover the above fee is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. 23-3050 in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 23-3050. A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Jeffrey J. King Woodcock Washburn LLP One Liberty Place - 46th Floor Philadelphia, PA 19103 (215) 568-3100					
 SIGNATURE				NAME	
Jeffrey J. King				38,515	
38,515				REGISTRATION NUMBER	

Rec'd PCT/PTO

03 MAY 2002

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DOCKET NO.: SMAR-0013

PATENT

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

In Re Application of:

Michael R. Hayden et a.

U.S. Serial No.: 10/019,341

Intl. Appln. No.: PCT/CA00/00762

Group Art Unit: not yet known

Intl. Filing Date: 23 June 2000

Examiner: not yet assigned

For: LPL VARIANT THERAPEUTICS

BOX PCT/RO/EO/US

Commissioner for Patents
United States Patent and Trademark Office
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U. S. Patent and Trademark Office

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**STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE
WITH 37 CFR §§ 1.821 THROUGH 1.825**

- ☒ I hereby state, in accordance with the requirements of 37 C.F.R. §1.821(f), that the contents of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR §1.821(c) and (e), respectively are the same.
- ☐ I hereby state that the submission filed in accordance with 37 CFR §1.821(g) does not include new matter.
- ☐ I hereby state that the submission filed in accordance with 37 CFR §1.821(h) does not include new matter or go beyond the disclosure in the international application as filed.
- ☐ I hereby state that the amendments, made in accordance with 37 CFR §1.825(a), included in the substitute sheet(s) of the Sequence Listing are supported in the application, as filed, at pages _____. I hereby state that the substitute sheet(s) of the Sequence Listing does (do) not include new matter.
- ☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with 37 CFR §1.825(b), is the same as the amended Sequence Listing.

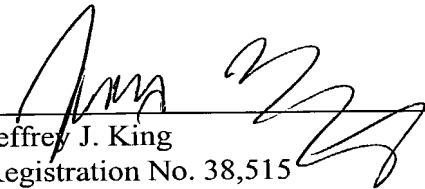
DOCKET NO.: SMAR-0013

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PATENT

☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with 37 CFR §1.825(d), contains identical data to that originally filed.

Date: May 3, 2002



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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
FOR THE PCT DESIGNATED/ELECTED OFFICE (PCT/DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 37**

In re national phase patent application of:

Michael R. HAYDEN and John J.P. KASTELEIN

International Application No.: PCT/CA00/00762

Serial No.: Not Yet Assigned

International Filing Date: 23 June 2000

Filing Date: Herewith

For: LPL VARIANT THERAPEUTICS

Assistant Commissioner of
Patents & Trademarks
Washington, DC 20231
Attn: Box PCT/DO/EO/US

Sir:

PRELIMINARY AMENDMENT

Prior to examination of the above-referenced patent application, please make the following amendments and consider the following remarks.

In the claims:

Please cancel current claims 1-34 and add the following new claims 35-57:

35. (Newly Added) A method of treating a disease in a subject, comprising administering to the subject an effective amount of an LPL S447X therapeutic, wherein the disease is an LPL-responsive disease.

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36. (Newly Added) The method of claim 35, wherein the LPL-responsive disease is selected from the group consisting of: chylomicronemia, hyperlipidemia, partial LPL deficiency, pancreatitis, hypertriglyceridemia, hypoalphalipoproteinemia (low HDL-cholesterol), cardiovascular disease, coronary heart disease, coronary artery disease, atherosclerosis, angina pectoris, hypertension, cerebrovascular disease, coronary restenosis, peripheral vascular disease, diabetes, cachexia and obesity.
37. (Newly Added) The method of claim 35, wherein the LPL-responsive disease is complete LPL deficiency.
38. (Newly Added) The method of claim 35, wherein the LPL S447X therapeutic is selected from the group consisting of:
- a) an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned; and,
 - b) an LPL S447X nucleic acid encoding the LPL S447X protein.
39. (Newly Added) The method of claim 38, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.
40. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.

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41. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.
42. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X protein, and the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.
43. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
44. (Newly Added) The method of claim 39, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
45. (Newly Added) The method of claim 40, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
46. (Newly Added) The method of claim 41, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.

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47. (Newly Added) The method of claim 43, wherein the gene therapy vector comprises a viral vector.
48. (Newly Added) The method of claim 44, wherein the gene therapy vector comprises a viral vector.
49. (Newly Added) The method of claim 45, wherein the gene therapy vector comprises a viral vector.
50. (Newly Added) The method of claim 46, wherein the gene therapy vector comprises a viral vector.
51. (Newly Added) The method of claim 35, wherein the subject is a human.
52. (Newly Added) A gene therapy vector comprising an LPL S447X nucleic acid encoding an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned.
53. (Newly Added) The gene therapy vector of claim 52, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.
54. (Newly Added) The gene therapy vector of claim 52, wherein the LPL S447X nucleic acid comprises a DNA coding sequence encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.

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55. (Newly Added) The gene therapy vector of claim 52, wherein the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.
56. (Newly Added) The gene therapy vector of claim 52, wherein the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.
57. (Newly Added) The gene therapy vector of claim 52, wherein the gene therapy vector comprises a viral vector.

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REMARKS

Early consideration and allowance of the above-referenced patent application is respectfully requested.

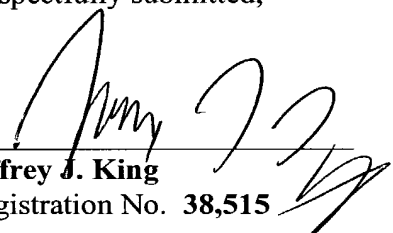
Claims 1-34 have been canceled. Claims 35-57 have been added.

The claims have been amended to remove multiple dependencies and to otherwise conform with U.S. claim practice. No new matter has been entered. None of the amendments change the scope of any claim, nor are any amendments submitted for reasons of patentability.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

Respectfully submitted,

Date: December 21, 2001



Jeffrey J. King
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DOCKET NO.: SMAR-0013

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claims 1-34 have been canceled.

Claims 35-57 have been added.

In the Claims:

35. (Newly Added) A method of treating a disease in a subject, comprising administering to the subject an effective amount of an LPL S447X therapeutic, wherein the disease is an LPL-responsive disease.
36. (Newly Added) The method of claim 35, wherein the LPL-responsive disease is selected from the group consisting of: chylomicronemia, hyperlipidemia, partial LPL deficiency, pancreatitis, hypertriglyceridemia, hypoalphalipoproteinemia (low HDL-cholesterol), cardiovascular disease, coronary heart disease, coronary artery disease, atherosclerosis, angina pectoris, hypertension, cerebrovascular disease, coronary restenosis, peripheral vascular disease, diabetes, cachexia and obesity.
37. (Newly Added) The method of claim 35, wherein the LPL-responsive disease is complete LPL deficiency.
38. (Newly Added) The method of claim 35, wherein the LPL S447X therapeutic is selected from the group consisting of:
- a) an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned; and,

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- b) an LPL S447X nucleic acid encoding the LPL S447X protein.
39. (Newly Added) The method of claim 38, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.
40. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.
41. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.
42. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X protein, and the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.
43. (Newly Added) The method of claim 38, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
44. (Newly Added) The method of claim 39, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.

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45. (Newly Added) The method of claim 40, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
46. (Newly Added) The method of claim 41, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
47. (Newly Added) The method of claim 43, wherein the gene therapy vector comprises a viral vector.
48. (Newly Added) The method of claim 44, wherein the gene therapy vector comprises a viral vector.
49. (Newly Added) The method of claim 45, wherein the gene therapy vector comprises a viral vector.
50. (Newly Added) The method of claim 46, wherein the gene therapy vector comprises a viral vector.
51. (Newly Added) The method of claim 35, wherein the subject is a human.
52. (Newly Added) A gene therapy vector comprising an LPL S447X nucleic acid encoding an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to

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SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned.

53. (Newly Added) The gene therapy vector of claim 52, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.

54. (Newly Added) The gene therapy vector of claim 52, wherein the LPL S447X nucleic acid comprises a DNA coding sequence encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.

55. (Newly Added) The gene therapy vector of claim 52, wherein the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.

56. (Newly Added) The gene therapy vector of claim 52, wherein the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.

57. (Newly Added) The gene therapy vector of claim 52, wherein the gene therapy vector comprises a viral vector.

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531 Rec'd PCT/P 21 DEC 2001

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LPL VARIANT THERAPEUTICS

FIELD OF THE INVENTION

5 The invention is in the field of protein and nucleic acid therapeutics based on variants of lipoprotein lipase (LPL), including therapeutics delivered by gene therapy.

BACKGROUND OF THE INVENTION

10 Lipoprotein lipase (EC 3.1.1.34) is an important enzyme in the metabolism of triglyceride-rich lipoproteins. It is synthesized in the parenchymal cells of adipose tissue and skeletal and cardiac muscle, where it is transferred to binding sites at the vascular side of endothelial cells on the vascular endothelium. Current understanding is that LPL plays an important role in the regulation of lipoprotein and lipid metabolism, as follows. The noncovalently-linked glycosylated homodimer is thought to be transported to the vascular endothelium, where it binds heparan

15 sulphate proteoglycans at the luminal surface. Subsequent catabolism of triglycerides from both chylomicrons (CM) and very low density lipoproteins (VLDL) is understood to allow the uptake and utilization of the free fatty acids and glycerol for energy and storage in muscle and adipose tissue respectively. Chylomicron and VLDL remnants may either be used in high density lipoprotein (HDL) or low density

20 lipoprotein (LDL) particle formation respectively, or taken up by the liver and repackaged into new VLDL particles. LPL has an obligatory requirement for its activator apolipoprotein (apo) CII, a small protein of 79 amino acids that is present on CM and VLDL particles. Inhibitors of LPL include free fatty acids, apo CIII, and possibly apo E. Another inhibitor is high concentrations of salt (1M NaCl).

25

 Although the cellular origin of LPL in the circulation is unclear, and may represent an accumulation from several tissue sources, its primary site of action is understood to be at the luminal surface of the vascular endothelium. Due to its non-covalent interaction with heparan sulphate proteoglycans, LPL may be displaced into

30 the plasma by an intravenous bolus injection of heparin. Thus, LPL activity and protein levels can be simply assessed by taking a small sample of post-heparin plasma (PHP). Aliquots of this PHP can then be used in either a synthetic, radiolabelled triglyceride (TG) assay for lipolytic activity or be measured by LPL

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specific antibodies for protein levels. Lipid measures may be performed in pre-heparin samples since the release of LPL may cause rapid lipolysis of the triglyceride in the sample.

- 5 Complete LPL deficiency occurs in approximately 1 in 10^6 persons, and the frequency is much higher in the French Canadian population where it may occur in up to 1 in 5000 individuals. The clinical manifestations of complete LPL deficiency in humans stem from infancy with a failure to thrive, colicky abdominal pain, hepatosplenomegaly, chylomicronemia characterized by lactescent plasma, eruptive
- 10 xanthomata, lipemia retinalis and life threatening pancreatitis. Lipid lowering drugs are ineffective and even rigid dietary restrictions are often poorly tolerated. The development of therapies for LPL deficiency would represent a major advance for persons suffering from this disorder.
- 15 Recently, patients with mutations in the LPL gene which result in partial defects in LPL catalytic function have been identified and, in fact, are very common in the general population. Collectively, known mutations resulting in partial catalytic defects in LPL are now estimated to occur with a frequency of between 5-7% in the general population. The clinical presentation may be quiescent, evident only by
- 20 marginally elevated triglyceride levels in the non-stressed state, with profound hypertriglyceridemia triggered by factors such as normal pregnancy, obesity or diabetes. Postprandial metabolic studies have been performed on individuals heterozygous for mutations in the LPL gene, demonstrating an unmasking of the lipolytic defect after a fat challenge, resulting in prolonged post-prandial lipemia and
- 25 significant disturbances in lipoprotein levels and composition. There is also evidence that specific mutations that alter, but do not abolish, LPL activity, such as Asn291Ser, Asp9Asn, exist commonly in the general population (Reymer et al., *Nat.Genet.* 1995, 10:28-33; Gagné et al., *Arterioscl.Thromb.* 1994, 14(8):1250-1257). The significance of this is not yet fully understood although they are
- 30 implicated in atherosclerosis susceptibility. A mutation that introduces a termination codon at position 447 in place of a serine codon (Ser447Ter or S447X) has been associated with decreased TG and increased HDL-cholesterol levels (Hokanson, 1997, *International Journal of Clinical and Laboratory Research* 27, 24-34; Gagné et

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al., *Arterioscl. Thromb.* 1994, 14(8):1250-1257; Mattu et al., 1994, *Arteriosclerosis and Thrombosis* 14, 1090-1097; Kuivenhoven et al., 1997, *Arteriosclerosis, Thrombosis and Vascular Biology* 17, 595-599; Groenemeijer et al., 1997, *Circulation* 95, 2628-2635; Fisher et al., 1997, *Atherosclerosis* 135, 145-159; U.S. Patent No. 5,658,729; Groenemeijer et al., *Circulation* 1997, 95:2628-2635; Gagne et al., *Clin. Genet.* 1999, 55(6):450-454). Correspondingly, in most studies this mutation seems to confer protection against CAD. The mechanism(s) behind these effects are not known.

10

SUMMARY OF THE INVENTION

One aspect of the invention involves the recognition of important advantages that may be obtained through therapeutic treatments comprising the administration of therapeutics derived from the LPL S447X protein and nucleic acid sequences encoding the LPL S447X protein. Such LPL S447X therapeutics may include LPL S447X peptides, nucleic acid sequences coding therefor, cells expressing such peptides or nucleic acids, and derivatives of such peptides, wherein the LPL S447X therapeutic ameliorates or treats disease when administered in prophylactically or therapeutically effective dosages. LPL S447X therapeutics of the invention include modifications, derivatives and analogs of LPL S447X peptides, and nucleic acids encoding such peptides. In some embodiments, the LPL S447X therapeutic of the invention may be a peptide having a sequence of amino acids corresponding to amino acids 1-446 of a naturally-occurring wild-type LPL peptide, as set out in FIG. 1 (SEQ ID NO: 1). A variety of naturally-occurring LPL peptides are known (Murthy V., Julien P., and Gagné C. 1996. *Molecular pathobiology of the human lipoprotein lipase gene. Pharmacol. Ther.* 70[2], 101-135). Alternative naturally-occurring LPL peptides may be identified by screening individual genomes, including non-human genomes, for sequences homologous to known LPL genes.

30

In alternative aspects, the invention provides for the use of an LPL S447X therapeutic, such as an LPL S447X protein or nucleic acid, for modulating LPL activity or LPL mass, for reducing plasma triglycerides and/or raising HDL-cholesterol, altering plasma lipid levels, or to treat an LPL-responsive condition in a

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patient. The invention also provides pharmaceutical compositions for such uses.

Examples of LPL-responsive conditions that may be amenable to treatment in alternative embodiments include: complete LPL deficiency (including chronic (e.g. for life, chylomicronemia, hypoalphalipoproteinemia) and acute (e.g. pancreatitis, severe hyperlipidemia) whether genetic or acquired); partial LPL deficiency (including chronic and acute (e.g. pancreatitis, hyperlipidemia, in pregnancy, diabetes, alcoholism); hyperlipidemia which is not due to LPL deficiency (e.g. FH, FCH, Type II lipoproteinemia); hypertriglyceridemia (having a variety of causes); hypoalphalipoproteinemia (low HDL), low HDL-cholesterol levels; cardiovascular disease; coronary heart disease; coronary artery disease; atherosclerosis; angina pectoris; hypertension (high blood pressure); cerebrovascular disease; coronary restenosis; peripheral vascular disease; diabetes (hypertriglyceridemia and other related symptoms in diabetes and insulin resistant states); cachexia (for example in cancer or when there is an altered LPL expression profile); and obesity.

In one aspect the invention relates to the use of an LPL S447X therapeutic selected from the group consisting of:

- a) an LPL S447X protein wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned; and,
- b) an LPL S447X nucleic acid encoding the LPL S447X protein.

In some embodiments, the LPL S447X protein may have an LPL activity or other therapeutic property equal to or greater than a wild-type LPL, such as the LPL of SEQ ID NO:3

In one aspect, the invention relates to the use in gene therapy of an LPL S447X nucleic acid encoding the LPL S447X protein. The LPL S447X nucleic acid may be delivered by a therapeutically acceptable gene therapy vector to treat LPL-responsive conditions, such as the conditions set out above. The gene therapy vector may for example be an adeno-associated vector (AAV). Such a vector may

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comprise for example: a 5' inverted terminal repeat (ITR); a promoter, such as a CMV enhancer-promoter with a muscle specific enhancer; an intron; a 3'-untranslated region (3'-UTR); a polyadenylation signal, such as an SV40 polyadenylation signal; and a 3'-ITR.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a listing of the amino acid sequence of LPL S447X, showing amino acids designated 1 through 446 herein (SEQ ID NO:1). The Figure reproduces information available from Genbank Accession NP_000228 for a *Homo sapiens* lipoprotein lipase precursor, version NP_000228.1, GI:4557727, incorporated herein by reference (Wion, et al., Science 235 (4796), 1638-1641 (1987); Sparkes et al., Genomics 1 (2), 138-144 (1987); Mattei et al., Cytogenet. Cell Genet. 63 (1), 45-46 (1993); Zechner, Curr. Opin. Lipidol. 8 (2), 77-88 (1997); Fisher et al., Atherosclerosis 135 (2), 145-159 (1997); and Beisiegel, Eur. Heart J. 19, A20-A23 (1998); Groenemeijer et al., Circulation 1997, 95:2628-2635; Gagne et al., Clin. Genet. 1999, 55(6):450-454).

Figure 2 is a listing of the amino acid sequence of a mature wild-type LPL peptide, showing amino acids designated 1 through 448 herein (SEQ ID NO:2). The Figure reproduces information available from Genbank Accession NP_000228.

Figure 3 is a listing of the amino acid sequence of a pre-LPL peptide, showing a protein having a signal peptide at amino acids 1 through 27, prior to the mature LPL peptide sequence (SEQ ID NO:3). The Figure reproduces information available from Genbank Accession NP_000228.

Figure 4 is a listing of the sequence of an LPL mRNA, in which a signal peptide is encoded by nucleotides 175 through 255, and the mature peptide is encoded by nucleotides 256 through 1599 (SEQ ID NO:4). The Figure reproduces information available from Genbank Accession NM_000237 for a *Homo sapiens* lipoprotein lipase (LPL) mRNA, version NM_000237.1, GI:4557726, incorporated herein by reference (Wion, et al., Science 235 (4796), 1638-1641 (1987); Sparkes et

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al., *Genomics* 1 (2), 138-144 (1987); Mattei et al., *Cytogenet. Cell Genet.* 63 (1), 45-46 (1993); Zechner, *Curr. Opin. Lipidol.* 8 (2), 77-88 (1997); Fisher et al., *Atherosclerosis* 135 (2), 145-159 (1997); and Beisiegel, *Eur. Heart J.* 19, A20-A23 (1998)).

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DETAILED DESCRIPTION OF THE INVENTION

In some embodiments, the LPL S447X therapeutic of the invention may include substantially purified compounds such as peptide fragments, modified

10 peptide fragments, analogues or pharmacologically acceptable salts of LPL having amino acids 447-448 truncated from the carboxy terminal of a wild-type LPL, such compounds are collectively referred to herein as LPL S447X peptides. LPL S447X peptides may include homologs of the wild-type mature LPL sequence from amino acids 1 through 446, including homologs from species other than *homo sapiens*

15 (which may have veterinary applications). LPL S447X peptides may include naturally occurring isoforms or genetic variants of wild type LPL. LPL polypeptides may also include polypeptides having substantial sequence similarity to wild type LPL amino acids 1 through 446, such as 90%, 95% or 99% sequence identity to a corresponding portion of the wild-type LPL 1-446 sequence, the corresponding

20 portion of wild-type LPL being any contiguous sequence of any length, such as 10, 20, 30, 40, 50 or more amino acids. In some embodiments, such proteins may have LPL activity, or another LPL-like property, equal to or greater than wild type LPL. In some embodiments, chemically similar amino acids may be substituted for amino acids in the wild-type LPL sequence (to provide conservative amino acid

25 substitutions). Amino acid substitutions that reduce LPL activity, of which more than 50 have been disclosed, such as the substitution of a Ser residue for Asn at position 291 (Asn291Ser), the substitution of Asn for Asp at position 9 (Asp9Asn), the substitution of Glu for Gly at position 188 (Gly188Glu, see Monsalve et al., *J.Clin.Invest.* 1990, 86(3):728-734) or Asp250Asn (Ma et al., *Genomics.* 1992,

30 13:649-653) may be avoided in preferred embodiments.

Two nucleic acid or protein sequences are considered substantially identical if, when optimally aligned, they share at least about 70% sequence identity. In alternative embodiments, sequence identity may for example be at least 75%, at

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least 90% or at least 95%. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms, such as the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl. Math* 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85: 2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence alignment may also be carried out using the BLAST algorithm, described in Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software for performing BLAST analysis may be available through the National Center for Biotechnology Information (through the internet at <http://www.ncbi.nlm.nih.gov/>). The BLAST algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold. Initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction is halted when the following parameters are met: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST programs may use as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (Henikoff and Henikoff, 1992, *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10 (which may be changed in alternative embodiments to 1 or 0.1 or 0.01 or 0.001 or 0.0001; although E values much higher than 0.1 may not identify functionally similar sequences, it is useful to examine hits with lower significance, E values between 0.1 and 10, for short regions of similarity), M=5, N=4, for nucleic acids a comparison of both strands. For protein comparisons, BLASTP may be used with defaults as follows: G=11 (cost to open a gap); E=1 (cost to extend a gap); E=10 (expectation value, at this setting, 10

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hits with scores equal to or better than the defined alignment score, S, are expected to occur by chance in a database of the same size as the one being searched; the E value can be increased or decreased to alter the stringency of the search.); and W=3 (word size, default is 11 for BLASTN, 3 for other blast programs).

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The BLOSUM matrix assigns a probability score for each position in an alignment that is based on the frequency with which that substitution is known to occur among consensus blocks within related proteins. The BLOSUM62 (gap existence cost = 11; per residue gap cost = 1; lambda ratio = 0.85) substitution matrix is used by default in BLAST 2.0. A variety of other matrices may be used as alternatives to BLOSUM62, including: PAM30 (9,1,0.87); PAM70 (10,1,0.87) BLOSUM80 (10,1,0.87); BLOSUM62 (11,1,0.82) and BLOSUM45 (14,2,0.87). One measure of the statistical similarity between two sequences using the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. In alternative embodiments of the invention, nucleotide or amino acid sequences are considered substantially identical if the smallest sum probability in a comparison of the test sequences is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

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It is well known in the art that some modifications and changes can be made in the structure of a polypeptide without substantially altering the biological function of that peptide, to obtain a biologically equivalent polypeptide. In one aspect of the invention, LPL S447X therapeutics may include peptides that differ from a portion of the wild-type LPL sequence by conservative amino acid substitutions. As used herein, the term "conserved amino acid substitutions" refers to the substitution of one amino acid for another at a given location in the peptide, where the substitution can be made without loss of function. In making such changes, substitutions of like amino acid residues can be made, for example, on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like, and such substitutions may be assayed for their effect on the function of the peptide by routine testing.

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In some embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0), where the following hydrophilicity values are assigned to amino acid residues (as detailed in United States Patent No. 4,554,101, incorporated herein by reference): Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4).

In alternative embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydropathic index (e.g., within a value of plus or minus 2.0). In such embodiments, each amino acid residue may be assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, as follows: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5).

In alternative embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another in the same class, where the amino acids are divided into non-polar, acidic, basic and neutral classes, as follows: non-polar: Ala, Val, Leu, Ile, Phe, Trp, Pro, Met; acidic: Asp, Glu; basic: Lys, Arg, His; neutral: Gly, Ser, Thr, Cys, Asn, Gln, Tyr.

The invention provides pharmaceutical compositions containing LPL S447X therapeutics. In some embodiments, such compositions may include a LPL S447X therapeutic in an effective amount, sufficient to provide a desired therapeutic or prophylactic effect, and a pharmaceutically acceptable carrier or excipient. An "effective amount" includes a therapeutically effective amount or a prophylactically effective amount.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as alteration of parameters in lipid metabolism, such as elevation of LPL activity,

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elevation of HDL-cholesterol or reduction of triglyceride levels. A therapeutically effective amount of LPL S447X therapeutic may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the LPL S447X therapeutic to elicit a desired response in the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. A therapeutically effective amount is also typically one in which any toxic or detrimental effects of the LPL S447X therapeutic are outweighed by the therapeutically beneficial effects.

A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, such as preventing or inhibiting various conditions, including LPL responsive conditions, such as coronary heart disease, cardiovascular disease, coronary artery disease, high triglycerides and/or low HDL. A prophylactic dose may be used in subjects prior to or at an earlier stage of disease, and a prophylactically effective amount may be more or less than a therapeutically effective amount in some cases.

In particular embodiments, a range for therapeutically or prophylactically effective amounts of LPL S447X therapeutic may be 0.01 nM-0.1M, 0.1 nM-0.1M, 0.1 nM-0.05M, 0.05 nM-15µM or 0.01 nM-10µM. It is to be noted that dosage values may vary with the severity of the condition to be alleviated. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need and the professional judgement of the person administering or supervising the administration of the compositions. Dosage ranges set forth herein are exemplary only and do not limit the dosage ranges that may be selected by medical practitioners.

For gene therapy vectors, the dosage to be administered may depend to a large extent on the condition and size of the subject being treated as well as the therapeutic formulation, frequency of treatment and the route of administration. Regimens for continuing therapy, including dose, formulation, and frequency may be guided by the initial response and clinical judgment. The parenteral route of injection into the interstitial space of tissue may be preferred, although other parenteral routes, such as inhalation of an aerosol formulation, may be required in specific administration. In some protocols, a formulation comprising the gene and gene

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delivery system in an aqueous carrier is injected into tissue in appropriate amounts.

The tissue target may be specific, for example the muscle or liver tissue, or it may be a combination of several tissues, for example the muscle and liver tissues.

Exemplary tissue targets may include liver, skeletal muscle, heart muscle, adipose
5 deposits, kidney, lung, vascular endothelium, epithelial and/or hematopoietic cells.

The amount of active compound in the compositions of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For
10 example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It may be advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. "Dosage unit form" as used herein refers to physically discrete
15 units suited as unitary dosages for subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention may be dictated by the unique characteristics of the active compound and the particular therapeutic effect to be
20 achieved, and by the limitations inherent in the art of compounding such an active compound for the treatment of a condition in individuals.

As used herein "pharmaceutically acceptable carrier" or "excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents,
25 isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal, intramuscular, sublingual or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the
30 extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the

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active compound, use thereof in the pharmaceutical compositions of the invention is contemplated.

Supplementary active compounds can also be incorporated into the
5 pharmaceutical compositions of the invention. For example, ApoCII (in nucleic acid or protein form) may in some embodiments act as a co-activator of an LPL S447X therapeutic. Alternative active compounds may include compounds that augment the properties or action of an LPL S447X therapeutic. In some embodiments the LPL S447X therapeutic may be co-administered with a therapeutic, such as insulin, for
10 treating an alternative condition, such as diabetes. In some embodiments, immune system modulators, such as cyclosporin, may be co-administered with the LPL S447X therapeutic, for example to ameliorate an immune response to the LPL S447X therapeutic. In assessing the risk of an immune response against an LPL S447X therapeutic, an analysis of the LPL gene of a patient may be carried out to
15 characterize the patient's natural LPL. Guidance on co-administration of additional therapeutics may for example be found in the Compendium of Pharmaceutical and Specialties (CPS) of the Canadian Pharmacists Association.

Pharmaceutical compositions are typically sterile and stable under the
20 conditions of manufacture and storage. Pharmaceutical compositions may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to accommodate high drug concentration. The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable
25 mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable
30 compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. The LPL S447X therapeutic may be administered in a time or controlled release formulation, for example in a composition which includes a slow release polymer or other carriers

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that will protect the compound against rapid release, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may for example be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. In accordance with an alternative aspect of the invention, an LPL S447X therapeutic may be formulated with one or more additional compounds that enhance the solubility of the LPL S447X therapeutic.

LPL S447X therapeutic compounds of the invention may include derivatives, such as C-terminal hydroxymethyl derivatives, O-modified derivatives (e.g., C-terminal hydroxymethyl benzyl ether), and N-terminally modified derivatives including substituted amides such as alkylamides and hydrazides.

Within an LPL S447X peptide of the invention, a peptidic structure maybe coupled directly or indirectly to a modifying group. The term "modifying group" is intended to include structures that are directly attached to the peptidic structure (e.g., by covalent coupling), as well as those that are indirectly attached to the peptidic structure (e.g., by a stable non-covalent association or by covalent coupling to additional amino acid residues, or mimetics, analogues or derivatives thereof, which may flank the MCP-3 core peptidic structure). For example, the modifying group can be coupled to the amino-terminus or carboxy-terminus of an LPL S447X peptidic structure, or to a peptidic or peptidomimetic region flanking the core domain.

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Alternatively, the modifying group can be coupled to a side chain of an amino acid residue of the LPL S447X peptide, or to a peptidic or peptido-mimetic region flanking the core domain (e.g., through the epsilon amino group of a lysyl residue(s), through the carboxyl group of an aspartic acid residue(s) or a glutamic acid residue(s),

5 through a hydroxy group of a tyrosyl residue(s), a serine residue(s) or a threonine residue(s) or other suitable reactive group on an amino acid side chain). Modifying groups covalently coupled to the peptidic structure can be attached by means and using methods well known in the art for linking chemical structures, including, for example, amide, alkylamino, carbamate or urea bonds.

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In some embodiments, the modifying group may comprise a cyclic, heterocyclic or polycyclic group. The term "cyclic group", as used herein, includes cyclic saturated or unsaturated (i.e., aromatic) group having from 3 to 10, 4 to 8, or 5 to 7 carbon atoms. Exemplary cyclic groups include cyclopropyl, cyclobutyl, 15 cyclopentyl, cyclohexyl, and cyclooctyl. Cyclic groups may be unsubstituted or substituted at one or more ring positions. A cyclic group may for example be substituted with halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, heterocycles, hydroxyls, aminos, nitros, thiols amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, sulfonates, selenoethers, 20 ketones, aldehydes, esters, -CF₃, -CN.

The term "heterocyclic group" includes cyclic saturated, unsaturated and aromatic groups having from 3 to 10, 4 to 8, or 5 to 7 carbon atoms, wherein the ring structure includes about one or more heteroatoms. Heterocyclic groups include 25 pyrrolidine, oxolane, thiolane, imidazole, oxazole, piperidine, piperazine, morpholine. The heterocyclic ring may be substituted at one or more positions with such substituents as, for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, aryls, other heterocycles, hydroxyl, amino, nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, 30 selenoethers, ketones, aldehydes, esters, -CF₃, -CN. Heterocycles may also be bridged or fused to other cyclic groups as described below.

The term "polycyclic group" as used herein is intended to refer to two or more saturated, unsaturated or aromatic cyclic rings in which two or more carbons are

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common to two adjoining rings, so that the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycyclic group may be substituted with such substituents as described above, as for example, halogens, alkyls, cycloalkyls, alkenyls, alkynyls, hydroxyl, amino, 5 nitro, thiol, amines, imines, amides, phosphonates, phosphines, carbonyls, carboxyls, silyls, ethers, thioethers, sulfonyls, selenoethers, ketones, aldehydes, esters, -CF₃, or -CN.

The term "alkyl" refers to the radical of saturated aliphatic groups, including 10 straight chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In some embodiments, a straight chain or branched chain alkyl has 20 or fewer carbon atoms in its backbone (C₁-C₂₀ for straight chain, C₃-C₂₀ for branched chain), or 10 or fewer carbon atoms. In some embodiments, cycloalkyls may have from 4-10 carbon atoms 15 in their ring structure, such as 5, 6 or 7 carbon rings. Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, having from one to ten carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have chain lengths of ten or less carbons.

20 The term "alkyl" (or "lower alkyl") as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, carbonyl (such as carboxyl, ketones (including 25 alkylcarbonyl and arylcarbonyl groups), and esters (including alkyloxycarbonyl and aryloxycarbonyl groups)), thiocarbonyl, acyloxy, alkoxyl, phosphoryl, phosphonate, phosphinate, amino, acylamino, amido, amidine, imino, cyano, nitro, azido, sulfhydryl, alkylthio, sulfate, sulfonate, sulfamoyl, sulfonamido, heterocyclyl, aralkyl, or an aromatic or heteroaromatic moiety. The moieties substituted on the 30 hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of aminos, azidos, iminos, amidos, phosphoryls (including phosphonates and phosphinates), sulfonyls (including sulfates, sulfonamidos, sulfamoyls and sulfonates), and silyl groups, as well as ethers, alkylthios, carbonyls (including

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pyridyl, pyrazinyl, pyridazinyl and pyrimidinyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, azide, alkyl, 5 aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, -CF₃, -CN, or the like. Aryl groups can also be part of a polycyclic group. For example, aryl groups include fused aromatic moieties such as naphthyl, anthracenyl, 10 quinolyl, indolyl, and the like.

Modifying groups may include groups comprising biotinyl structures, fluorescein-containing groups, a diethylene-triaminepentaacetyl group, a (-)-menthoxyacetyl group, a N-acetylneuraminyl group, a cholyl structure or an 15 iminiobiotinyl group. An LPL S447X peptide may be modified at its carboxy terminus with a cholyl group according to methods known in the art (for example see: Wess, G. et al. (1993) Tetrahedron Letters, 34:817-822; Wess, G. et al. (1992) Tetrahedron Letters 33:195-198; and Kramer, W. et al. (1992) J. Biol. Chem. 267:18598-18604). Cholyl derivatives and analogues may also be used as modifying groups, such as 20 Aic (3-(O-aminoethyl-iso)-cholyl), which has a free amino group that can be used to further modify the LPL S447X peptide. A modifying group may be a "biotinyl structure", which includes biotinyl groups and analogues and derivatives thereof (such as a 2-iminobiotinyl group). In another embodiment, the modifying group may comprise a "fluorescein-containing group", such as a group derived from reacting an 25 LPL S447X peptide with 5-(and 6-)-carboxyfluorescein, succinimidyl ester or fluorescein isothiocyanate. In various other embodiments, the modifying group(s) may comprise an N-acetylneuraminyl group, a trans-4-cotininecarboxyl group, a 2-imino-1-imidazolidineacetyl group, an (S)-(-)-indoline-2-carboxyl group, a (-)-menthoxyacetyl group, a 2-norbornaneacetyl group, a gamma-oxo-5-acenaphthenebutyryl, a (-)-2-oxo-4-thiazolidinecarboxyl group, a tetrahydro-3-furoyl 30 group, a 2-iminobiotinyl group, a diethylenetriaminepentaacetyl group, a 4-morpholinecarbonyl group, a 2-thiopheneacetyl group or a 2-thiophenesulfonyl group.

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An LPL S447X therapeutic of the invention may be further modified to alter the specific properties of the compound while retaining the desired functionality of the compound. For example, in one embodiment, the compound may be modified to alter a pharmacokinetic property of the compound, such as *in vivo* stability or half-life. The compound may be modified to label the compound with a detectable substance. The compound may be modified to couple the compound to an additional therapeutic moiety. To further chemically modify the compound, such as to alter its pharmacokinetic properties, reactive groups can be derivatized. Potential C-terminal modifications include those which reduce the ability of the compound to act as a substrate for carboxypeptidases. Examples of C-terminal modifiers include an amide group, an ethylamide group and various non-natural amino acids, such as D-amino acids and beta-alanine. Alternatively, when the modifying group is attached to the carboxy-terminal end of the aggregation core domain, the amino-terminal end of the compound may be further modified, for example, to reduce the ability of the compound to act as a substrate for aminopeptidases.

An LPL S447X therapeutic can be further modified to label the compound by reacting the compound with a detectable substance. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{14}C , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{99}Tc , ^{35}S or ^3H . An LPL S447X therapeutic may be radioactively labelled with ^{14}C , either by incorporation of ^{14}C into the modifying group or one or more amino acid structures in the compound. Labelled LPL S447X therapeutics may be used to assess the *in vivo* pharmacokinetics of the compounds, as well as to detect disease progression or propensity of a subject to develop a disease, for example for diagnostic purposes. Tissue distribution of LPL S447X therapeutics can be detected using a labeled LPL S447X therapeutic either *in vivo* or

In an alternative chemical modification, an LPL S447X therapeutic of the invention may be prepared in a "prodrug" form, wherein the compound itself does not act as a therapeutic, but rather is capable of being transformed, upon metabolism *in vivo*, into an LPL S447X therapeutic as defined herein. For example, in this type of compound, the modifying group can be present in a prodrug form that is capable of being converted upon metabolism into the form of an active LPL S447X therapeutic. Such a prodrug form of a modifying group is referred to herein as a "secondary modifying group." A variety of strategies are known in the art for preparing peptide prodrugs that limit metabolism in order to optimize delivery of the active form of the peptide-based drug (see e.g., Moss, J. (1995) in *Peptide-Based Drug Design: Controlling Transport and Metabolism*, Taylor, M. D. and Amidon, G. L. (eds), Chapter 18).

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Milligen/Bioscience 9600). Peptides may be assayed for activity in accordance with standard methods. Peptides may be purified by HPLC and analyzed by mass spectrometry. Peptides may be dimerized via a disulfide bridge formed by gentle oxidation of the cysteines using 10% DMSO in water. Following HPLC purification

5 dimer formation may be verified, by mass spectrometry. One or more modifying groups may be attached to an LPL S447X peptide by standard methods, for example using methods for reaction through an amino group (e.g., the alpha-amino group at the amino-terminus of a peptide), a carboxyl group (e.g., at the carboxy terminus of a peptide), a hydroxyl group (e.g., on a tyrosine, serine or threonine residue) or other

10 suitable reactive group on an amino acid side chain (see e.g., Greene, T. W. and Wuts, P. G. M. Protective Groups in Organic Synthesis, John Wiley and Sons, Inc., New York (1991)).

In another aspect of the invention, LPL S447X peptides may be prepared

15 according to standard recombinant DNA techniques using a nucleic acid molecule encoding the peptide. A nucleotide sequence encoding the peptide of interest may be determined using the genetic code and an oligonucleotide molecule having this nucleotide sequence may be synthesized by standard DNA synthesis methods (e.g., using an automated DNA synthesizer). Alternatively, a DNA molecule encoding a

20 peptide compound may be derived from the natural precursor protein gene or cDNA (e.g., using the polymerase chain reaction (PCR) and/or restriction enzyme digestion) according to standard molecular biology techniques. For example, the human wild type LPL cDNA fragment may be cloned by RT-PCR from human

adipose tissue total RNA using the following 5' and 3' UTR primers respectively; 5'-

25 ATA GAA TTC GGA TCC ATC GAT/GC TCC TCC AGA GGG ACG GCG CCC CG-3' (which introduces an EcoRI, BamHI and ClaI site 5' of the LPL coding sequence) and 5'-TAT GTC GAC TAG ATA TC/GCC GTT CTT TGT TCT GTA GAT TCG CCC-3' (introducing Sall, XbaI and EcoRV sites 3' of the LPL coding sequence). The LPL S447X cDNAs may be derived from the wild type human 1.6kb LPL cDNA by site

30 directed mutagenesis (which may be confirmed by sequencing, see Henderson et al., 1991, Journal of Clinical Investigation 87, 2005-2011; and, Zhang et al., 1996Biochimica et Biophysica Acta 1302, 159-166).

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The invention also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding an LPL S447X peptide of the invention. The term "nucleic acid molecule" includes DNA molecules and RNA molecules which may be single-stranded or double-stranded. In alternative embodiments, the isolated nucleic acid encodes a peptide wherein one or more amino acids are altered or deleted.

To facilitate expression of a peptide compound in a host cell by standard recombinant DNA techniques, the isolated nucleic acid encoding the peptide may be incorporated into a recombinant expression vector. Accordingly, the invention also provides recombinant expression vectors comprising the nucleic acid molecules of the invention. As used herein, the term "vector" refers to a nucleic acid, protein, lipid or other molecule capable of transporting a nucleic acid to which it has been operatively linked. Vectors may include circular double stranded DNA plasmids and viral vectors. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (such as bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (such as non-episomal mammalian vectors) may be integrated into the genome of a host cell upon introduction into the host cell, and thereby may be replicated along with the host genome. Certain vectors may be capable of directing the expression of genes to which they are operatively linked.

In recombinant vectors of the invention, the nucleotide sequence encoding a peptide may be operatively linked to one or more regulatory sequences, selected on the basis of the host cells to be used for expression. The terms "operatively linked" or "operably" linked mean that the sequences encoding the peptide are linked to the regulatory sequence(s) in a manner that allows for expression of the peptide compound. The term "regulatory sequence" includes promoters, enhancers, polyadenylation signals and other expression control elements. Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) (incorporated herein by reference). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell, those that direct expression of the nucleotide sequence only in certain host cells (such as tissue-specific regulatory sequences) and those that direct expression in a

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regulatable manner (such as only in the presence of an inducing agent). The design of the expression vector may depend on such factors as the choice of the host cell to be transformed and the level of expression of peptide compound desired.

5 Recombinant expression vectors of the invention may be designed for expression of peptide compounds in prokaryotic or eukaryotic cells. For example, peptide compounds may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in*
10 *Enzymology* 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector may be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al., (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943),
15 pJRY88 (Schultz et al., (1987) *Gene* 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, Calif.). Baculovirus vectors available for expression of proteins or peptides in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al., (1983) *Mol. Cell. Biol.* 3:2156-2165) and the pVL series (Lucklow, V. A., and Summers, M. D., (1989) *Virology* 170:31-39). Examples of mammalian
20 expression vectors include pCDM8 (Seed, B., (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987), *EMBO J.* 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

25 In addition to regulatory control sequences, recombinant expression vectors may contain additional nucleotide sequences, such as a selectable marker gene to identify host cells that have incorporated the vector. Selectable marker genes are well known in the art. To facilitate secretion of the peptide compound from a host
30 cell, in particular mammalian host cells, the recombinant expression vector preferably encodes a signal sequence operatively linked to sequences encoding the amino-terminus of the peptide compound, such that upon expression, the peptide compound is synthesised with the signal sequence fused to its amino terminus. This signal sequence directs the peptide compound into the secretory pathway of the cell

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and is then cleaved, allowing for release of the mature peptide compound (i.e., the peptide compound without the signal sequence) from the host cell. Use of a signal sequence to facilitate secretion of proteins or peptides from mammalian host cells is well known in the art.

5

A recombinant expression vector comprising a nucleic acid encoding a peptide compound may be introduced into a host cell to produce the peptide compound in the host cell. Accordingly, the invention also provides host cells containing the recombinant expression vectors of the invention. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell may be any prokaryotic or eukaryotic cell. For example, a peptide compound may be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells. The peptide compound may be expressed *in vivo* in a subject to the subject by gene therapy (discussed further below).

20

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection" refer to techniques for introducing foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, microinjection and viral-mediated transfection. Suitable methods for transforming or transfecting host cells can for example be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other laboratory manuals. Methods for introducing DNA into mammalian cells *in vivo* are also known, and may be used to deliver the vector DNA of the invention to a subject for gene therapy.

30

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (such as resistance to

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antibiotics) may be introduced into the host cells along with the gene of interest.

Preferred selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acids encoding a selectable marker may be introduced into a host cell on the same vector as that encoding the peptide
 5 compound or may be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid may be identified by drug selection (cells that have incorporated the selectable marker gene will survive, while the other cells die).

A nucleic acid of the invention may be delivered to cells *in vivo* using methods
 10 such as direct injection of DNA, receptor-mediated DNA uptake, viral-mediated transfection or non-viral transfection and lipid based transfection, all of which may involve the use of gene therapy vectors. Direct injection has been used to introduce naked DNA into cells *in vivo* (see e.g., Acsadi et al. (1991) Nature 332:815-818; Wolff et al. (1990) Science 247:1465-1468). A delivery apparatus (e.g., a "gene
 15 gun") for injecting DNA into cells *in vivo* may be used. Such an apparatus may be commercially available (e.g., from BioRad). Naked DNA may also be introduced into cells by complexing the DNA to a cation, such as polylysine, which is coupled to a ligand for a cell-surface receptor (see for example Wu, G. and Wu, C. H. (1988) J. Biol. Chem. 263:14621; Wilson et al. (1992) J. Biol. Chem. 267:963-967; and U.S.
 20 Pat. No. 5,166,320). Binding of the DNA-ligand complex to the receptor may facilitate uptake of the DNA by receptor-mediated endocytosis. A DNA-ligand complex linked to adenovirus capsids which disrupt endosomes, thereby releasing material into the cytoplasm, may be used to avoid degradation of the complex by intracellular lysosomes (see for example Curiel et al. (1991) Proc. Natl. Acad. Sci. USA 88:8850;
 25 Cristiano et al. (1993) Proc. Natl. Acad. Sci. USA 90:2122-2126).

Defective retroviruses are well characterized for use as gene therapy vectors (for a review see Miller, A. D. (1990) Blood 76:271). Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses
 30 can be found in Current Protocols in Molecular Biology, Ausubel, F. M. et al. (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE and pEM which are well known to those skilled in the art. Examples of suitable packaging virus lines include .psi.Crip, .psi.Cre, .psi.2 and .psi.Am. Retroviruses have been

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used to introduce a variety of genes into many different cell types, including epithelial cells, endothelial cells, lymphocytes, myoblasts, hepatocytes, bone marrow cells, in vitro and/or in vivo (see for example Eglitis, et al. (1985) Science 230:1395-1398; Danos and Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson et al. 5 (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al. (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber et al. (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al. (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al. (1991) Science 254:1802-1805; van Beusechem et al. (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay et al. (1992) Human Gene Therapy 3:641- 10 647; Dai et al. (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu et al. (1993) J. Immunol. 150:4104-4115; U.S. Pat. No. 4,868,116; U.S. Pat. No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573).

15 For use as a gene therapy vector, the genome of an adenovirus may be manipulated so that it encodes and expresses a peptide compound of the invention, but is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. See for example Berkner et al. (1988) BioTechniques 6:616; Rosenfeld et al. (1991) Science 252:431-434; and Rosenfeld et al. (1992) Cell 68:143-155. Suitable 20 adenoviral vectors derived from the adenovirus strain Ad type 5 dl324 or other strains of adenovirus (e.g., Ad2, Ad3, Ad7 etc.) are well known to those skilled in the art. Recombinant adenoviruses are advantageous in that they do not require dividing cells to be effective gene delivery vehicles and can be used to infect a wide variety of cell types, including airway epithelium (Rosenfeld et al. (1992) cited supra), 25 endothelial cells (Lemarchand et al. (1992) Proc. Natl. Acad. Sci. USA 89:6482-6486), hepatocytes (Herz and Gerard (1993) Proc. Natl. Acad. Sci. USA 90:2812-2816) and muscle cells (Quantin et al. (1992) Proc. Natl. Acad. Sci. USA 89:2581-2584).

30 Adeno-associated virus (AAV) may be used as a gene therapy vector for delivery of DNA for gene therapy purposes. AAV is a naturally occurring defective virus that requires another virus, such as an adenovirus or a herpes virus, as a helper virus for efficient replication and a productive life cycle (Muzyczka et al. Curr. Topics in Micro. and Immunol. (1992) 158:97-129). AAV may be used to integrate

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DNA into non-dividing cells (see for example Flotte et al. (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski et al. (1989) J. Virol. 63:3822-3828; and McLaughlin et al. (1989) J. Virol. 62:1963-1973). An AAV vector such as that described in Tratschin et al. (1985) Mol. Cell. Biol. 5:3251-3260 may be used to introduce DNA
5 into cells (see for example Hermonat et al. (1984) Proc. Natl. Acad. Sci. USA 81:6466-6470; Tratschin et al. (1985) Mol. Cell. Biol. 4:2072-2081; Wondisford et al. (1988) Mol. Endocrinol. 2:32-39; Tratschin et al. (1984) J. Virol. 51:611-619; and Flotte et al. (1993) J. Biol. Chem. 268:3781-3790). Lentiviral gene therapy vectors may also be adapted for use in the invention.

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General methods for gene therapy are known in the art. See for example, U.S. Pat. No. 5,399,346 by Anderson *et al.* (incorporated herein by reference). A biocompatible capsule for delivering genetic material is described in PCT Publication WO 95/05452 by Baetge *et al.* Methods of gene transfer into hematopoietic cells
15 have also previously been reported (see Clapp, D. W., et al., Blood 78: 1132-1139 (1991); Anderson, Science 288:627-9 (2000); and , Cavazzana-Calvo *et al.*, Science 288:669-72 (2000), all of which are incorporated herein by reference).

Genetic LPL deficiency may be classified into three categories depending on
20 the LPL protein characteristics. Type I hypertriglyceridemic patients have very low to absent LPL protein mass. Type II patients produce little pre-heparin LPL protein but the level increases following heparin treatment. In class Type III, there are large amounts of circulating pre-heparin protein with little change in levels after heparin challenge. Although the utility of this classification system is limited, with some
25 compound heterozygote patients spanning two of these classes, clarification of the presence or absence of plasma LPL protein may be important for deciding which patients are most likely to tolerate gene transfer without an immune reaction to LPL S447X therapeutics, with the spectrum being from Type I patients, being the most likely to develop an immune reaction, to Type II patients, being the least likely to
30 develop an immune reaction.

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EXAMPLE 1**Administration of LPL S447X Protein by Gene Therapy**

A mouse model of human LPL deficiency has been created by gene-targeting to inactivate the murine LPL gene. All homozygous (-/-) pups die within 48 hours after birth with massive chylomicronemia from suckling milk. In attempt to rescue the -/- pups, intramuscular delivery of recombinant adenovirus containing the wild type human LPL gene (Ad-LPL) alone did not result in significant increase of human LPL mass in post-heparin plasma and did not rescue the lethality of complete LPL deficiency.

Intramuscular delivery of an Ad-447 gene therapy vector to newborn pups resulted in the appearance of human LPL mass in high levels in post-heparin plasma. In a litter of 4 pups, 2 were injected with Ad-447 (2×10^8 pfu in 100 μ l PBS) to 4 sites (25 μ l/site) in 4 limbs at the day of birth. Two days later heparin at 1000 u/kg was injected intraperitoneally and the pups were sacrificed by decapitation to collect about 10 to 20 μ l post-heparin plasma for analysis. One front and one hind limb were harvested and homogenized at 100 mg/ml in extraction buffer. The Results are shown in Table 1.

Table 1

	<u>Cont'l</u>	<u>Ad-447</u>
LPL mass in post-heparin plasma:	61.9	5351.2
(ng/ml)	61.0	8992.6
LPL activity in muscle homogenate:	43.2	215.6
(mU/ml)	35.8	119.7
LPL mass in muscle homogenate:	29.5	1820.7
(ng/ml)	45.3	1478.7

These results show that intramuscular delivery of a LPL S447X therapeutic by adenoviral-mediated gene transfer resulted in significant increase of human LPL protein mass in post-heparin plasma. These results are indicative of the surprisingly advantageous results obtainable using the LPL S447X therapeutics of the invention, as compared to wild type LPL.

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An adenovirus vector of the invention for gene therapy may for example be produced utilizing the Adeno-Quest™ kit available from Quantum Biotechnologies Inc. (Montreal, QC, Canada). In an example of this approach, cDNAs were cloned into the shuttle plasmid pQBI-AdCMV5, which contains the CMV5 promoter and enhancer and a globin poly A. The LPL cDNAs (wild type and S447X) were double digested with HindIII and XbaI and inserted into the BamHI site via blunt end ligation. The human LPL gene was inserted 1.5 map units (mu) downstream from the 5' end of the adenovirus genome and is followed by mu 9.4-15.5 of Ad5 allowing for homologous recombination. The shuttle vector and the right end of the *Clal*-digested, E3-deleted fragment of the Ad5 genome were co-transfected in 293 cells by calcium-phosphate precipitation and overlaid with 0.8% agarose in DMEM/5% FBS. After plaque purification, a high expressing clone was selected and amplified. At day 9 and 14 plaques were screened *in vitro* for LPL activity. Two clones with the highest LPL activity activity were chosen, plaque purified a second time and amplified in 293A cell culture on 15 cm plates. Purification of high titer recombinant virus (~3x10¹⁰ pfu/ml) was performed by double rounds of CsCl density gradient ultracentrifugation. The purified virus stocks were dialyzed against 4 changes of HEPES buffered saline (HBS, 20mM Hepes, 150 mM NaCl, pH 7.3) with 10% glycerol over 16-18 hours and stored at -80°C. Titers of the viral stocks were determined by plaque assay using 293 cells as described above. The titer was calculated as plaque forming units per milliliter (pfu/ml) and was 1-3 x 10¹⁰ pfu/ml. Virus preparations were quantified by Lowry protein assay consistently revealed ~50 particles per pfu from all Ad-LPL and Ad-447 preparations.

EXAMPLE 2

A serotype 5 adenovirus containing a LPL S447X gene under the control of the CMV-promoter was developed (Ad-447 as described in Example 1), and its effect compared to the effects of a wildtype LPL containing adenovirus. The animal model employed was the +/- LPL knock-out mouse model (Coleman et al., 1995, The Journal of Biological Chemistry 270[21], 12518-12525).

Preliminary *in vitro* studies done in HepG2 cells indicated a dose response relationship for LPL activity for the Ad-447 virus of a similar magnitude to the

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adenovirus containing the wild type LPL. There was, however, a marked difference in the amount of LPL immunoreactive mass. LPL immunoreactive mass in Ad-447 treated cells, at an MOI of 50 which infects essentially 100% of the cells, was roughly 4-fold greater than that of Ad-LPL.

5

When the dose response relationship of the Ad-447 virus was evaluated in a small cohort of mice via intravenous injection, unexpected results were obtained. The level of LPL activity was not responsive to 2 fold increases of virus (5×10^8 , 1×10^9 or 2×10^9 pfu/mouse). The level of activity was similar to that seen in the cohort of mice given 5×10^8 pfu of Ad-LPL. However, the level of LPL protein seen in these mice was exceptionally high. Despite activity apparently starting to decrease at day 7, the immunoreactive mass continued to increase at all three doses to a level of approximately 35-40,000 ng/ml. The majority of this protein at day 7 was observed in pre-heparin plasma and the small variation in lipolytic activity indicated that it was largely in an inactive form. Approximately 5000ng/ml was found exclusively in post-heparin plasma. Over a time course of 70 days, LPL activity levels in mice given Ad-447 followed those of Ad-LPL closely and returned to baseline levels between 6 and 10 weeks post-injection. However, LPL immunoreactive protein levels were significantly elevated in mice receiving either Ad-LPL or Ad-447 with levels in the Ad-447 cohort maintaining profoundly elevated levels over the wild type Ad-LPL group. TG levels were significantly reduced at all three doses of Ad-447 in a manner similar to 5×10^8 pfu Ad-LPL. Both total and HDL cholesterol were significantly reduced until day 14.

25

To demonstrate dose response, LPL +/- mice (n=5/group) were given either 5×10^7 or 5×10^8 pfu of either Ad-447 or Ad-LPL (a dose 5×10^7 pfu is equivalent to approximately 5×10^9 particles). At a dose of 5×10^8 pfu of either Ad-LPL or Ad-447 per mouse, there was a significant 2.7 fold increase in plasma LPL activity accompanied by a significant increase in LPL protein levels at day 5 post gene transfer. Corresponding TG, HDL-C and Total-C levels also dropped significantly. At this dose, the only significant difference between the adenovector containing the wild type LPL cDNA versus the Ad-447 was the plasma LPL protein level, which was elevated in the Ad-447 group. Although significantly elevated in both groups over

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20 This example illustrates that LPL mass and activity is associated with the severity of ischaemia and angina pectoris, indicating that the S447X therapeutics of the present invention may be used to treat such conditions by elevating LPL mass or activity.

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with LPL-mass ($r=0.70$; $p<0.0001$). A significant association between the LPL-protein mass and NYHA class ($p=0.012$) was also demonstrated. These results demonstrate a significant relationship between LPL mass and activity and severity of ischaemia as defined by angina class and AECG, indicating that LPL S447X therapeutics that

5 adjust effective LPL mass or activity may be used to treat ischaemia and angina pectoris.

EXAMPLE 4

This example illustrates that LPL S447X protein is associated with protection

10 against coronary heart disease, indicating that the S447X therapeutics of the present invention may be used to treat this condition. A total of 1114 men and 1144 women were randomly ascertained from the Framingham Offspring Study (FOS) for the presence of the LPL S447X gene. Carrier frequency of the LPL S447X allele was 17%, and in men carrier status was associated with higher total cholesterol (TC)

15 ($\Delta=6.2$ mg/dl, $p=0.03$), higher HDL-C ($\Delta=2.3$ mg/dl, $p=0.01$) and lower triglyceride (TG) levels ($\Delta=-19.4$ mg/dl, $p=0.02$). Moreover, in men the LPL S447X allele conferred significant protection against CHD (odds ratio: 0.43; $p=0.04$).

EXAMPLE 5

20 This example demonstrates the utility of LPL S447X therapeutics in an alternative model of human disease. The animal model employed was the completely deficient (-/-) ApoE knock-out mouse model. The therapeutic effect of a serotype 5 adenovirus containing an LPL S447X gene under the control of the CMV-promoter (Ad-447 virus) was compared to the therapeutic effect of a wildtype LPL-

25 containing adenovirus (Ad-LPL), as well as to a control alkaline phosphatase (AP) containing adenovirus (Ad-AP).

Intravenous tail vein injection of 5×10^8 pfu of Ad-447 or wild type Ad-LPL resulted in a large reduction of plasma TG levels and a large increase in both LPL

30 activity and protein mass levels in post-heparin plasma, in comparison to control injected Ad-AP mice, revealing the surprising efficacy of LPL S447X therapeutics in alternative therapies involving modulation of LPL activity or mass.

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Table 2

Virus	N	Pre-heparin Plasma			Post-heparin Plasma	
		TG (mg/dl)	HDL-C (mg/dl)	T-C (mg/dl)	LPL Activity (mU/ml)	LPL Mass (ng/ml)
Control	2	34 ± 7	12 ± 1	192 ± 1	784 ± 222	13 ± 22
Ad-447	1	0	14	178	1395	9810
Ad-LPL	2	5 ± 3	13 ± 4	178 ± 3	1335 ± 39	5511 ± 3447

5

Table 2 illustrates LPL and lipid measures 3 days after the administration of 5x10⁸ pfu of Ad-LPL, Ad-447 or control Alkaline Phosphatase to ApoE deficient mice. In agreement with data obtained in heterozygous LPL deficient mice, plasma triglycerides were decreased while both LPL activity and protein mass levels were increased in mice receiving Ad-LPL or Ad-447, with Ad-447 providing comparatively greater TG reduction, increased LPL activity and very significant LPL mass enhancement.

15

An exemplary protocol for assaying the effect of intravenous and intramuscular delivery of an LPL S447X therapeutic in an animal disease model are as follows. A serotype 5 adenovirus containing an LPL S447X gene under the control of the CMV-promoter (Ad-447) may be compared to the effects of a wildtype LPL containing adenovirus, Ad-LPL. The animal model may be the +/- LPL knock-out mouse model (Coleman et al., 1995, The Journal of Biological Chemistry 270[21], 12518-12525). For example, a total dose of 5x10⁸ pfu may be diluted into 120ul with a sterile saline solution and divided into 4 equal parts. A volume of 30ul may be injected directly into the tibialis anterior and the gastrocnemius of both legs of each mouse for a total dose of 5x10⁸ pfu per mouse. Blood may be obtained at days 3 and 7 post-treatment and the muscle categories may be isolated 14 days post-treatment for tissue analysis.

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CONCLUSION

Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such

5 modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. In the specification, the word "comprising" is used as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding

10 meaning. Citation of references herein shall not be construed as an admission that such references are prior art to the present invention. All publications, including but not limited to patents and patent applications, cited in this specification are incorporated herein by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein and as though fully

15 set forth herein.

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WHAT IS CLAIMED IS:

1. The use of an LPL S447X therapeutic for the preparation of a pharmaceutical composition for the treatment of an LPL-responsive condition in a subject.
2. The use of an LPL S447X therapeutic according to claim 1, wherein the LPL-responsive condition is selected from the group consisting of: complete LPL deficiency, chylomicronemia, hyperlipidemia, partial LPL deficiency, pancreatitis, hypertriglyceridemia, hypoalphalipoproteinemia (low HDL-cholesterol), cardiovascular disease, coronary heart disease, coronary artery disease, atherosclerosis, angina pectoris, hypertension, cerebrovascular disease, coronary restenosis, peripheral vascular disease, diabetes, cachexia and obesity.
3. The use of an LPL S447X therapeutic according to claim 1 or 2, wherein the LPL S447X therapeutic is selected from the group consisting of:
 - a) an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned; and
 - b) an LPL S447X nucleic acid encoding the LPL S447X protein.
4. The use of an LPL S447X therapeutic according to claim 3, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.
5. The use of the LPL S447X therapeutic according to claim 3 or 4, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence

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encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.

- 5 6. The use of the LPL S447X therapeutic according to claim 3 or 4, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.
- 10 7. The use of the LPL S447X therapeutic according to claim 3 or 4, wherein the LPL S447X therapeutic is the LPL S447X protein, and the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.
- 15 8. The use of the LPL S447X therapeutic according to claim 3, 4, 5 or 6, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the pharmaceutical composition comprises a gene therapy vector.
- 20 9. The use of the LPL S447X therapeutic according to claim 8, wherein the gene therapy vector comprises a viral vector.
10. The use of the LPL S447X therapeutic according to any one of claims 1 through 9, wherein the subject is a human.
- 25 11. A method of treating a disease in a subject, comprising administering to the subject an effective amount of an LPL S447X therapeutic, wherein the disease is an LPL-responsive disease.
- 30 12. The method of claim 11, wherein the LPL-responsive disease is selected from the group consisting of: complete LPL deficiency, chylomicronemia, hyperlipidemia, partial LPL deficiency, pancreatitis, hypertriglyceridemia, hypoalphalipoproteinemia (low HDL-cholesterol), cardiovascular disease, coronary heart disease, coronary artery

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disease, atherosclerosis, angina pectoris, hypertension, cerebrovascular disease, coronary restenosis, peripheral vascular disease, diabetes, cachexia and obesity.

- 5 13. The method of claim 11 or 12, wherein the LPL S447X therapeutic is selected from the group consisting of:
 - 10 a) an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned; and,
 - b) an LPL S447X nucleic acid encoding the LPL S447X protein.
- 15 14. The method of claim 13, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.
- 20 15. The method of claim 13 or 14, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.
- 25 16. The method of claim 13 or 14, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.
- 30 17. The method of claim 13 or 14, wherein the LPL S447X therapeutic is the LPL S447X protein, and the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.

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18. The method of any one of claims 13, 14, 15 or 16, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X therapeutic is administered to the patient in a gene therapy vector.
- 5 19. The method of claim 18, wherein the gene therapy vector comprises a viral vector.
20. The method of any one of claims 11 through 19, wherein the subject is a human.
- 10 21. An LPL S447X therapeutic for use as an active pharmaceutical substance, wherein the LPL S447X therapeutic is selected from the group consisting of:
- 15 a) an LPL S447X protein, wherein the amino acid sequence of the LPL S447X protein comprises a contiguous segment having at least 90% sequence identity to SEQ ID NO:3 when optimally aligned, and wherein the LPL S447X protein lacks amino acids corresponding to amino acids 447 and 448 of SEQ ID NO:3 when optimally aligned; and,
- 20 b) an LPL S447X nucleic acid encoding the LPL S447X protein.
22. The LPL S447X therapeutic according to claim 21, wherein the LPL S447X protein has greater LPL activity than a wild type LPL of SEQ ID NO:3.
- 25 23. The LPL S447X therapeutic according to claim 21 or 22, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence encoding an RNA having at least 90% sequence identity to nucleotides 256 through 1599 of SEQ ID NO:4.
- 30 24. The LPL S447X therapeutic according to claim 21 or 22, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes

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31. The gene therapy vector of claim 28 or 29, wherein the LPL S447X therapeutic is the LPL S447X nucleic acid, and the LPL S447X nucleic acid comprises a DNA coding sequence that hybridizes under stringent conditions to nucleotides 256 through 1599 of SEQ ID NO:4.
32. The gene therapy vector of claim 28 or 29, wherein the LPL S447X therapeutic is the LPL S447X protein, and the contiguous segment has at least 95% sequence identity to SEQ ID NO:1.
33. The gene therapy vector of any one of claims 28 through 32, wherein the gene therapy vector comprises a viral vector.
34. An LPL S447X therapeutic substantially as hereinbefore described and with reference to the examples and drawings.

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- Published:**
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **LPL VARIANT THERAPEUTICS****LPL S447X 1-446 Peptide:**

```

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aesvatchfn hssktfmvih gwtvtgmyes wvpklvaaly krepdsnviv vdwlszagen 93
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```

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(57) Abstract: The invention provides for the use of a therapeutic derived from a truncated lipoprotein lipase protein (LPL S447X), including nucleic acids encoding such proteins, for the treatment of conditions including LPL responsive conditions, such as cardiovascular disease, hypertension, LPL deficiency, high triglyceride levels, low HDL-cholesterol levels or atherosclerosis.

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Figure 1

LPL S447X 1-446 Peptide:

```

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gldpagpnfe yaeapsrlsp ddadfdvdlh tftgrspgrs igiqkpvghv diypnggtfq 213
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lclscrknrc nnlgyeinkv rakrsskmyl ktrsqmpykv fhyqvkihfs gtesethtnq 333
afeislygtv aesenipftl pevstnktys fliytevdig ellmlklkwk sdsyfswsdw 393
wsspgfaiqk irvkaetqk kvifcsrekv shlqkgkapa vfvkchdksl nkk 446

```

Figure 2

Mature LPL Peptide 1-448:

```

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afeislygtv aesenipftl pevstnktys fliytevdig ellmlklkwk sdsyfswsdw 393
wsspgfaiqk irvkaetqk kvifcsrekv shlqkgkapa vfvkchdksl nkksg 448

```

Figure 3

Pre-LPL Peptide: 1-27 Signal Peptide (boxed) and Mature Peptide 28-475:

```

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301 lclscrknrc nnlgyeinkv rakrsskmyl ktrsqmpykv fhyqvkihfs gtesethtnq
361 afeislygtv aesenipftl pevstnktys fliytevdig ellmlklkwk sdsyfswsdw
421 wsspgfaiqk irvkaetqk kvifcsrekv shlqkgkapa vfvkchdksl nkksg

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Figure 4

LPL mRNA

```

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Figure 4 Continued

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DOCKET NO. SMAR-0013

COPY

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:

Michael R. HAYDEN and John J. P. KASTELEIN **Group Art Unit:** Not Yet Assigned**International Application No.:** PCT/CA00/00762 **Examiner:** Not Yet Assigned**International Filing Date:** 23 June 2000**For:** LPL VARIANT THERAPEUTICS

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a

☒ Utility Patent ☐ Design Patent

is sought on the invention, whose title appears above, the specification of which:

- ☐ is attached hereto.
- ☒ was filed on 23 June 2000 as
International Application No. PCT/CA00/00762 .
- ☒ said application having been amended on August 20, 2001 .

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with 37 CFR § 1.56.

DOCKET NO. SMAR-0013

- 2 -

PATENT

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a-d) of any **foreign application(s)** for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of any application on which priority is claimed:

Priority Claimed (If X'd)	Country	Serial Number	Date Filed
<input checked="" type="checkbox"/>	EP	99202048.7	24 June 1999
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I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Date Filed	Patented/Pending/Abandoned

S00S Y/M

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- 3 -

PATENT

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Serial Number

Date Filed

_____	_____
_____	_____

I hereby appoint the following persons of the firm of **WOODCOCK WASHBURN LLP**, One Liberty Place - 46th Floor, Philadelphia, Pennsylvania 19103 as attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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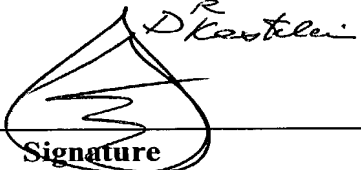
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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- 4 -

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Mailing Address: Hauwert 141 NL-1691 EE Hauwert THE NETHERLANDS	Date of Signature: 11-3-2002
City/State of Actual Residence: Hauwert, THE NETHERLANDS	Citizenship: Netherlands

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SEQUENCE LISTING

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Asn Arg Ile Thr Gly Leu Asp Pro Ala Gly Pro Asn Phe Glu Tyr Ala			
	180	185	190
Glu Ala Pro Ser Arg Leu Ser Pro Asp Asp Ala Asp Phe Val Asp Val			
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Leu His Thr Phe Thr Arg Gly Ser Pro Gly Arg Ser Ile Gly Ile Gln			
	210	215	220
Lys Pro Val Gly His Val Asp Ile Tyr Pro Asn Gly Gly Thr Phe Gln			
	225	230	235
Pro Gly Cys Asn Ile Gly Glu Ala Ile Arg Val Ile Ala Glu Arg Gly			
	245	250	255
Leu Gly Asp Val Asp Gln Leu Val Lys Cys Ser His Glu Arg Ser Ile			
	260	265	270
His Leu Phe Ile Asp Ser Leu Leu Asn Glu Glu Asn Pro Ser Lys Ala			
	275	280	285
Tyr Arg Cys Ser Ser Lys Glu Ala Phe Glu Lys Gly Leu Cys Leu Ser			
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Cys Arg Lys Asn Arg Cys Asn Asn Leu Gly Tyr Glu Ile Asn Lys Val			
	305	310	315
Arg Ala Lys Arg Ser Ser Lys Met Tyr Leu Lys Thr Arg Ser Gln Met			
	325	330	335
Pro Tyr Lys Val Phe His Tyr Gln Val Lys Ile His Phe Ser Gly Thr			
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Glu Ser Glu Thr His Thr Asn Gln Ala Phe Glu Ile Ser Leu Tyr Gly			
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Thr Val Ala Glu Ser Glu Asn Ile Pro Phe Thr Leu Pro Glu Val Ser			
	370	375	380
Thr Asn Lys Thr Tyr Ser Phe Leu Ile Tyr Thr Glu Val Asp Ile Gly			
	385	390	395
			400

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Thr	Asn	Lys	Lys	Val	Asn	Arg	Ile	Thr	Gly	Leu	Asp	Pro	Ala	Gly	Pro	145	150	155	160
Asn	Phe	Glu	Tyr	Ala	Glu	Ala	Pro	Ser	Arg	Leu	Ser	Pro	Asp	Asp	Ala	165	170	175	
Asp	Phe	Val	Asp	Val	Leu	His	Thr	Phe	Thr	Arg	Gly	Ser	Pro	Gly	Arg	180	185	190	
Ser	Ile	Gly	Ile	Gln	Lys	Pro	Val	Gly	His	Val	Asp	Ile	Tyr	Pro	Asn	195	200	205	
Gly	Gly	Thr	Phe	Gln	Pro	Gly	Cys	Asn	Ile	Gly	Glu	Ala	Ile	Arg	Val	210	215	220	
Ile	Ala	Glu	Arg	Gly	Leu	Gly	Asp	Val	Asp	Gln	Leu	Val	Lys	Cys	Ser	225	230	235	240
His	Glu	Arg	Ser	Ile	His	Leu	Phe	Ile	Asp	Ser	Leu	Leu	Asn	Glu	Glu	245	250	255	
Asn	Pro	Ser	Lys	Ala	Tyr	Arg	Cys	Ser	Ser	Lys	Glu	Ala	Phe	Glu	Lys	260	265	270	
Gly	Leu	Cys	Leu	Ser	Cys	Arg	Lys	Asn	Arg	Cys	Asn	Asn	Leu	Gly	Tyr	275	280	285	
Glu	Ile	Asn	Lys	Val	Arg	Ala	Lys	Arg	Ser	Ser	Lys	Met	Tyr	Leu	Lys	290	295	300	
Thr	Arg	Ser	Gln	Met	Pro	Tyr	Lys	Val	Phe	His	Tyr	Gln	Val	Lys	Ile	305	310	315	320
His	Phe	Ser	Gly	Thr	Glu	Ser	Glu	Thr	His	Thr	Asn	Gln	Ala	Phe	Glu	325	330	335	
Ile	Ser	Leu	Tyr	Gly	Thr	Val	Ala	Glu	Ser	Glu	Asn	Ile	Pro	Phe	Thr	340	345	350	
Leu	Pro	Glu	Val	Ser	Thr	Asn	Lys	Thr	Tyr	Ser	Phe	Leu	Ile	Tyr	Thr	355	360	365	
Glu	Val	Asp	Ile	Gly	Glu	Leu	Leu	Met	Leu	Lys	Leu	Lys	Trp	Lys	Ser	370	375	380	
Asp	Ser	Tyr	Phe	Ser	Trp	Ser	Asp	Trp	Trp	Ser	Ser	Pro	Gly	Phe	Ala	385	390	395	400

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Ile Gln Lys Ile Arg Val Lys Ala Gly Glu Thr Gln Lys Lys Val Ile
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Kastelein, John J.P.

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Phe Met Val Ile His Gly Trp Thr Val Thr Gly Met Tyr Glu Ser Trp
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Val Pro Lys Leu Val Ala Ala Leu Tyr Lys Arg Glu Pro Asp Ser Asn
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Val Ile Val Val Asp Trp Leu Ser Arg Ala Gln Glu His Tyr Pro Val
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Ser Ala Gly Tyr Thr Lys Leu Val Gly Gln Asp Val Ala Arg Phe Ile
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Asn Trp Met Glu Glu Glu Phe Asn Tyr Pro Leu Asp Asn Val His Leu
115 120 125

Leu Gly Tyr Ser Leu Gly Ala His Ala Ala Gly Ile Ala Gly Ser Leu
130 135 140

Thr Asn Lys Lys Val Asn Arg Ile Thr Gly Leu Asp Pro Ala Gly Pro
145 150 155 160

Asn Phe Glu Tyr Ala Glu Ala Pro Ser Arg Leu Ser Pro Asp Asp Ala
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Asp Phe Val Asp Val Leu His Thr Phe Thr Arg Gly Ser Pro Gly Arg ;
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 Ser Ile Gly Ile Gln Lys Pro Val Gly His Val Asp Ile Tyr Pro Asn
 195 200 205
 Gly Gly Thr Phe Gln Pro Gly Cys Asn Ile Gly Glu Ala Ile Arg Val
 210 215 220
 Ile Ala Glu Arg Gly Leu Gly Asp Val Asp Gln Leu Val Lys Cys Ser
 225 230 235 240
 His Glu Arg Ser Ile His Leu Phe Ile Asp Ser Leu Leu Asn Glu Glu
 245 250 255
 Asn Pro Ser Lys Ala Tyr Arg Cys Ser Ser Lys Glu Ala Phe Glu Lys
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 Gly Leu Cys Leu Ser Cys Arg Lys Asn Arg Cys Asn Asn Leu Gly Tyr
 275 280 285
 Glu Ile Asn Lys Val Arg Ala Lys Arg Ser Ser Lys Met Tyr Leu Lys
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 Thr Arg Ser Gln Met Pro Tyr Lys Val Phe His Tyr Gln Val Lys Ile
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 His Phe Ser Gly Thr Glu Ser Glu Thr His Thr Asn Gln Ala Phe Glu
 325 330 335
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 Glu Val Asp Ile Gly Glu Leu Leu Met Leu Lys Leu Lys Trp Lys Ser
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 Asp Ser Tyr Phe Ser Trp Ser Asp Trp Trp Ser Ser Pro Gly Phe Ala
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35 40 45

Thr Ala Glu Asp Thr Cys His Leu Ile Pro Gly Val Ala Glu Ser Val
50 55 60

Ala Thr Cys His Phe Asn His Ser Ser Lys Thr Phe Met Val Ile His
65 70 75 80

Gly Trp Thr Val Thr Gly Met Tyr Glu Ser Trp Val Pro Lys Leu Val
85 90 95

Ala Ala Leu Tyr Lys Arg Glu Pro Asp Ser Asn Val Ile Val Val Asp
100 105 110

Trp Leu Ser Arg Ala Gln Glu His Tyr Pro Val Ser Ala Gly Tyr Thr
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Lys Leu Val Gly Gln Asp Val Ala Arg Phe Ile Asn Trp Met Glu Glu
130 135 140

Glu Phe Asn Tyr Pro Leu Asp Asn Val His Leu Leu Gly Tyr Ser Leu
145 150 155 160

Gly Ala His Ala Ala Gly Ile Ala Gly Ser Leu Thr Asn Lys Lys Val
165 170 175

Asn Arg Ile Thr Gly Leu Asp Pro Ala Gly Pro Asn Phe Glu Tyr Ala
180 185 190

Glu Ala Pro Ser Arg Leu Ser Pro Asp Asp Ala Asp Phe Val Asp Val
195 200 205

Leu His Thr Phe Thr Arg Gly Ser Pro Gly Arg Ser Ile Gly Ile Gln
210 215 220

Lys Pro Val Gly His Val Asp Ile Tyr Pro Asn Gly Gly Thr Phe Gln
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Pro Gly Cys Asn Ile Gly Glu Ala Ile Arg Val Ile Ala Glu Arg Gly
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Leu Gly Asp Val Asp Gln Leu Val Lys Cys Ser His Glu Arg Ser Ile
260 265 270

His Leu Phe Ile Asp Ser Leu Leu Asn Glu Glu Asn Pro Ser Lys Ala
275 280 285

Tyr Arg Cys Ser Ser Lys Glu Ala Phe Glu Lys Gly Leu Cys Leu Ser
290 295 300

Cys Arg Lys Asn Arg Cys Asn Asn Leu Gly Tyr Glu Ile Asn Lys Val
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Arg Ala Lys Arg Ser Ser Lys Met Tyr Leu Lys Thr Arg Ser Gln Met
325 330 335

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Glu Ser Glu Thr His Thr Asn Gln Ala Phe Glu Ile Ser Leu Tyr Gly
355 360 365

Thr Val Ala Glu Ser Glu Asn Ile Pro Phe Thr Leu Pro Glu Val Ser
370 375 380

Thr Asn Lys Thr Tyr Ser Phe Leu Ile Tyr Thr Glu Val Asp Ile Gly
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Glu Leu Leu Met Leu Lys Leu Lys Trp Lys Ser Asp Ser Tyr Phe Ser
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Trp Ser Asp Trp Trp Ser Ser Pro Gly Phe Ala Ile Gln Lys Ile Arg
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Val Lys Ala Gly Glu Thr Gln Lys Lys Val Ile Phe Cys Ser Arg Glu
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Val Ala Glu Ser Val Ala Thr Cys His Phe Asn His Ser Ser Lys Thr
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Phe Met Val Ile His Gly Trp Thr Val Thr Gly Met Tyr Glu Ser Trp
50 55 60

Val Pro Lys Leu Val Ala Ala Leu Tyr Lys Arg Glu Pro Asp Ser Asn

65 70 75 80
 Val Ile Val Val Asp Trp Leu Ser Arg Ala Gln Glu His Tyr Pro Val
 85 90 95
 Ser Ala Gly Tyr Thr Lys Leu Val Gly Gln Asp Val Ala Arg Phe Ile
 100 105 110
 Asn Trp Met Glu Glu Glu Phe Asn Tyr Pro Leu Asp Asn Val His Leu
 115 120 125
 Leu Gly Tyr Ser Leu Gly Ala His Ala Ala Gly Ile Ala Gly Ser Leu
 130 135 140
 Thr Asn Lys Lys Val Asn Arg Ile Thr Gly Leu Asp Pro Ala Gly Pro
 145 150 155 160
 Asn Phe Glu Tyr Ala Glu Ala Pro Ser Arg Leu Ser Pro Asp Asp Ala
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 Asp Phe Val Asp Val Leu His Thr Phe Thr Arg Gly Ser Pro Gly Arg
 180 185 190
 Ser Ile Gly Ile Gln Lys Pro Val Gly His Val Asp Ile Tyr Pro Asn
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 Gly Gly Thr Phe Gln Pro Gly Cys Asn Ile Gly Glu Ala Ile Arg Val
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 Ile Ala Glu Arg Gly Leu Gly Asp Val Asp Gln Leu Val Lys Cys Ser
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 His Glu Arg Ser Ile His Leu Phe Ile Asp Ser Leu Leu Asn Glu Glu
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 Asn Pro Ser Lys Ala Tyr Arg Cys Ser Ser Lys Glu Ala Phe Glu Lys
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 Gly Leu Cys Leu Ser Cys Arg Lys Asn Arg Cys Asn Asn Leu Gly Tyr
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 Glu Ile Asn Lys Val Arg Ala Lys Arg Ser Ser Lys Met Tyr Leu Lys
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Glu Val Asp Ile Gly Glu Leu Leu Met Leu Lys Leu Lys Trp Lys Ser
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Asp Ser Tyr Phe Ser Trp Ser Asp Trp Trp Ser Ser Pro Gly Phe Ala
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Ile Gln Lys Ile Arg Val Lys Ala Gly Glu Thr Gln Lys Lys Val Ile
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